## REMARKS

### Status of the Claims

Claims 1-2, 5-7, 9, 13-18, 21-22 and 24 are pending in the present application. Claims 1, 2, 5, 7, 9, 13, 16, 17, 18, 21, 22 and 24 are amended without prejudice or disclaimer. Claims 3, 4, 8, 10, 11, 12, 19, 20, 23 and 25 are canceled. Claims 15, 17, 22 and 24 are withdrawn as directed to a non-elected invention. Claims 1 and 2 are amended to specify "a mouse with Fas function defects" and to further specify that the glypican protein is a human glypican protein. Claim 7 is amended to specify that the immunized animal is a mouse. Support for these elements is found throughout the application as originally filed including, *e.g.*, in original claims 4 and 5 and page 3, lines 1-6. Claims 5, 9, 17, 21, 22 and 24 are amended to depend from a pending claim. Claim 13 is amended to cancel the autoimmune diseases as described above. Claims 16 and 18 are amended to depend from claim 7 and for consistency with claim 7. No new matter is entered by way of this amendment. Reconsideration is respectfully requested.

### Claim Objections

Claim 13 and 23 are objected to by the Examiner, *see* Office Action, page 2. The Examiner states that the term "erythematodes" is not an English word. The Examiner suggests that the correct term is "erythematosous." In addition, the Examiner states that the term thyroiditis and autoimmune are misspelled in claim 13.

Claim 23 is canceled. Accordingly, the objection is moot in regard to this claim. The claims are amended according to the Examiner's suggestions and to correct the spelling errors. Applicants respectfully request withdrawal of the objections.

### Objections to the Specification

The specification is objected to under 35 U.S.C. § 132 as allegedly incorporating new matter, *see* Office Action, page 2. Specifically, the Examiner states that the substitute sequence listing, which was submitted to correct a typographical error, describes LLV at amino acid positions 10-12 in SEQ ID NO: 6. The Examiner states that this recitation is incorrect. The

Examiner asserts that the correct amino acids at positions 10-12 of SEQ ID NO: 6 should be LVV. The Examiner further states that Figure 1 in the originally filed application lists amino acids 10-12 as "LVV" not as "LLV" as described in the substitute sequence listing, *see* Office Action, page 2. Applicants respectfully traverse.

SEQ ID NO: 6 is a mouse sequence. In contrast, the amino acid sequence at positions 11-12 of Figure 1 to which the Examiner refers is a human sequence. The amino acid sequence at positions 10-12 varies between mouse and human. The mouse sequence, which is shown in Figure 1 beneath the human sequence, depicts the amino acid sequences at positions 10-12 as LLV not LVV as the Examiner asserts. Accordingly, Applicants respectfully submit that the amendment filed on January 29, 2008, does not introduce new matter into the application. Withdrawal of the objection is respectfully requested.

**Issues Under 35 U.S.C. § 112, First Paragraph**

*Written Description*

Claims 1-9, 13-14, 16, 18-21 and 23-25 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement, *see* Office Action, pages 3-4. Specifically, the Examiner states that, at the time of filing, Applicants were in possession of a method for producing antibodies against GPC-3 protein comprising immunizing an MLR/lpr mouse that develops SLE with a GPC-3 protein, *see* Office Action, page 3. However, the Examiner alleges that Applicants were not in possession of the methods described in claims 1-9, 13, 14, 16, 18-21, 23 and 25. Specifically, the Examiner states that the instant application does not describe a representative number of species or structural features that are common to the antigen genus described in the claims.

According to the Examiner, the claims appear to encompass the use of any non-human animal that develops autoimmune disease and any human antigen that has a sequence identity of 94% or more at the amino acid sequence level to the homolog of the protein of the non-human animal to be immunized. The Examiner further states that Applicants have only disclosed glypican-3 protein and MLR/lpr mouse. Therefore, according to the Examiner, an ordinary artisan cannot envision all the contemplated human native proteins, which have a sequence

identity of 94% or more at the amino acid sequence level to a homolog protein of the non-human animals to be immunized or any non-human animal that develops autoimmune disease. Applicants respectfully traverse.

Claim 3, 4, 19, 20, 23 and 25 are canceled. Accordingly, the rejection is moot in regard to these claims.

In order to comply with the written description requirement, "[t]he applicant must...convey to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention." *Vas-Cath. v. Mahurkar*, 935 F.2d 1555, 1563-64 (Fed. Cir. 1991). The descriptive text needed to meet these requirements "varies with the nature and scope of the invention at issue, and with the scientific knowledge already in existence." *Capon v. Eshhar*, 418 F.3d 1349,1357, 76 USPQ2d 1078, 1084 (Fed. Cir. 2005).

In *Capon*, both parties to a patent interference appealed the decision of the Board of Patent Appeals and Interferences, which held that the claims of the parties failed to meet the written description requirement of 35 U.S.C. § 112, first paragraph. The claims at issue were directed to chimeric genes designed to enhance immune response using known DNA sequences of known function. The Court of Appeals for the Federal Circuit held that the Board erred in holding that the specifications do not meet the written description requirement because they do not reiterate the structure or formula of possible DNA components of the chimeric genes. The court stated that the Boards' requirement that the sequences of the component DNA be reported in the specification does not add descriptive substance. A person experienced in the field of the invention would understand that the known DNA segments retain their DNA sequences when linked by known methods. *See Capon*, 418 F 3d at 1349, 76 USPQ2d at 1085.

As amended, claim 1 is directed to a process for producing an antibody against a glypican protein comprising immunizing a mouse with Fas function defects that develops autoimmune disease with a human glypican protein. Claim 2, as amended, is directed to a process for producing an antibody against a glypican protein comprising immunizing an autoantibody-producing mouse with Fas function defects with a human glypican protein. Claim 7, as amended, is directed to a process for producing an antibody comprising immunizing a mouse

with Fas function defects with a human native protein which has high sequence identity of 94% or more at the amino acid sequence level to a homolog protein of the mouse to be immunized.

Similar to the facts in *Capon*, the instant claims describe a genus of sequences which were known in the art at the time of the invention for use with a novel, non-obvious method. The genus of sequences described in independent claim 7, *i.e.*, human native proteins having 94% or more sequence identity at the amino acid sequence level to a homolog protein of the mouse to be immunized, could have been envisioned by an ordinary artisan at the time of filing. Sequences of human and mouse proteins were available at the time of the invention from any number of art-known sequence databases. In addition, the instant application teaches, *e.g.*, on page 8, lines 6-10, a description of sequence identity and methods for determining sequence identity. Further, independent claims 1, 2 and 7, are amended in an effort to expedite prosecution to specify a mouse with Fas function defects. Accordingly, the claims are not directed to any non-human animal. Based upon the foregoing, Applicants submit that the instant claims comply with the written description requirement. Accordingly, Applicants respectfully request withdrawal of the rejection.

*Enablement*

Claims 1-9, 13-14, 16, 18-21, 23 and 25 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement, *see* Office Action, pages 4-6. Specifically, the Examiner states that the present application enables a method for producing an antibody against GPC-3 protein comprising immunizing a MLR/lpr mouse that develops SLE with a GPC-3 protein. However, the Examiner asserts that the present application fails to provide an enabling disclosure for producing an antibody, including an anti-glypican antibody, using any non-human animal that develops autoimmune disease, including those having Fas function defects. According to the Examiner, the instant application fails to show that any non-human animal model can be used in the production of antibodies against non-self antigens. In addition, the Examiner states that creating transgenic/knockout non-human mammals in different species of transgenic animals is unpredictable. The Examiner further states that the genotype and phenotype of a non-human animal that develops an autoimmune disease, including Fas function defects, may significantly vary, depending upon the genes that are manipulated.

Claims 3, 4, 19, 20, 23 and 25 are canceled. Accordingly, the rejection is moot in regard to these claims.

Although Applicants do not agree with the Examiner, in an effort to expedite prosecution independent claims 1, 2, and 7 are amended to specify "mouse" in lieu of "non-human animal." The independent claims further specify that the mouse has Fas function defects. An ordinary artisan recognizes that a mouse with Fas function defects encompasses a mouse with a mutated lpr gene, as described in the instant examples, as well as a mouse having a gld mutated gene. An ordinary artisan further recognizes that the lpr mutated gene and the gld mutated gene encode Fas and FasL, (which is a ligand of Fas), respectively. Further, an ordinary artisan recognizes that mice with gld gene mutations have similar features to those with lpr mutations, *see* Exhibit A, *i.e.*, Cohen *et* al., *Annu. Rev. Immunology*, 1991, 9:243-269, at page 257-258, enclosed. Based upon the foregoing, Applicants submit that the present application supports the instant claims. Accordingly, withdrawal of the rejection is respectfully requested.

**Issues Under 35 U.S.C. § 102(b)**

Claims 7-9, 14, 16, 18 and 19 are rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by U.S. Patent No. 6,235,714 to Paul *et al.* ("'714"), *see* Office Action, pages 6-8. The Examiner states that the '714 patent describes MRL/lpr mice that were hyperimmunized with target antigen, such as EGFR, TNFα, and IL-1β to drive the immune system to generate catalytic antibodies. In addition, the Examiner alleges that the antigen proteins described in the '714 patent are highly homologous between human and mouse. In particular, the Examiner states that the '714 patent discloses a protein having 100% sequence identity with a mouse protein antigen having the PKKKMEK sequence of Figure 14. Applicants respectfully traverse.

Applicants previously argued that the '714 patent teaches that PKKKMEK, *i.e.*, CRAA-IL1-β peptide, has three essential elements and corresponds to the formula: X1-Y-E-X2. Applicants previously stated that X1 and X2 are peptide sequences containing about 3-10 contiguous amino acids that form an epitope of a target antigen. Y is a basic residue (Arg or Lys). E is an electrophilic reaction center designed to react covalently with nucleophilic side chains of certain amino acids. Accordingly, Applicants stated that the '714 patent describes, as

an essential feature, a requirement that E must be present and adjacent to Y in order to obtain antibodies. In response, the Examiner states that independent claim 7 encompasses such antigens since this claim specifies the open-ended term "has."

Applicants also previously argued that the '714 patent describes a method that can only be used with insoluble antigens. In response, the Examiner states that the '714 patent describes injections with the soluble extracellular domain of the epidermal growth factor receptor (exEGFR). The Examiner alleges that this teaching indicates that the '714 patent describes a method that allows for the use of soluble antigens.

Claim 19 is canceled. Accordingly, the rejection is moot in regard to this claim.

Claim 7 specifies that the immunogen is a human native protein. Applicants submit that human native proteins do not encompass the PKKKMEK sequence. The electrophilic reaction center, which is positioned on the PKKKMEK sequence, is disclosed in the '714 patent as a phosphonic ester. Such an electrophilic reaction center is not present in a human native protein. Accordingly, even if the phrase "a human native protein which has high sequence identity of 94% or more at the amino acid sequence level" specifies the open-ended term "has", human native proteins never contain the electrophilic reaction center. Accordingly, claim 7 is not anticipated by the '714 patent.

Applicants further submit that the '714 patent does not describe the use of soluble antigens. As noted above, the Examiner states that the '714 patent discloses soluble proteins since the patent teaches that further injections with the soluble extracellular domain of exEGFR are administered to an animal. However, the '714 patent states "[i]f high levels Ab titers are not reached [after the immunization of A431 cells], booster injections with the soluble extracellular domain of the epidermal growth factor receptor (exEGFR)(25 .mu.g) will be administered," *see* column 14, lines 52-54. Accordingly, the '714 patent teaches that the immunization of A431 cells does not elicit antibodies. The '714 patent simply does not describe whether or not a desired antibody can be obtained by immunization with exEGFR. Instead, the '714 patent teaches that TSA-EGFR, conjugated with KLH, is immunized to a MRL/lpr mouse, such that the immune system generates a catalytic antibody. That is, the '714 patent merely discloses that TSA-EGFR should is used to generate catalytic antibodies.

Further, even if, hypothetically, the '714 patent suggests that an antibody could possibly be obtained from a MRL/lpr mouse that is immunized with an immunogen having the electrophilic reaction center, the '714 patent does not demonstrate that the immunization of the human native protein, EGFR or exEGFR, elicits an antibody. Moreover, the percentage of sequence identity of EGFR or exEGFR between a human and a mouse is 78%. The '714 patent does not demonstrate that immunization with an antigen having such a low sequence identity is capable of eliciting an antibody. Given that the field of immunology is unpredictable, the '714 patent does not teach or suggest that antibodies can be generated using an immunogen, which shares a sequence identity between mouse and human greater than of EGFR or exEGFR. Based upon the foregoing, Applicants submit that the instant claims are not anticipated by the '714 patent. Accordingly, withdrawal of the rejection is respectfully requested.

**Issues Under 35 U.S.C. § 103(a)**

*Claims 1-4, 6, 13, 20 and 23*

Claims 1-4, 6, 13, 20 and 23 are rejected under 35 U.S.C. § 103(a) as allegedly being obvious over JP-01047390 to Yamazaki Mashiko et al., ("390") or U.S. Patent No. 4,965,198 to Yamasaki *et al.*, ("198") each in view of Lage *et al.*, *Virchows Arch*, 2001, 438:567-573, ("Lage."). *see* Office Action, pages 8-9. Specifically, the Examiner states that the '390 publication and the '198 patent teach that it is preferable to immunize a mouse having an autoimmune disease, such as an NZB mouse, with an antigen having low immunogenicity, such as a glycolipid. The Examiner admits that the '390 publication and/or the '198 patent do not describe glypican. However, the Examiner states that Lage describes glypican-3 and further teaches that the immunogenicity of glypican-3 is low. According to the Examiner, it would have been obvious to one of ordinary skill in the art at the time of the invention to make monoclonal antibodies against glypican-3 using autoimmune mice. The Examiner asserts that an ordinary artisan would have been motivated to combine these references to solve a well known problem in the art, *i.e.*, the difficulty in producing mAb that specifically recognizes the weakly immunogenic or non-immunogenic GPC3 in mice. Applicants respectfully traverse.

Claims 3, 4 and 23 are canceled. Accordingly, the rejection is moot in regard to this claim.

Applicants submit that the '390 publication and the '198 patent teach away from the instant invention. The '198 patent and the '390 publication disclose that, when N-glycolyneuraminic acid containing glycolipids are used an immunogen, a mouse having an autoimmune disease is preferably used to produce antibodies. However, the '198 patent and the '390 publication further describe that a normal mouse, such as a Balb/c mouse, which has been administered bacterial lipopolysaccharide (LPS), or the like, to enhance the production of autoantibodies, and is in an autoimmune disease state, may also be used as an immunized animal. That is, the '198 patent and the '390 publication suggest that either a Balb/c mouse, administered with LPS, or the like, or a mouse having an autoimmune disease, such as an NZB mouse, are equally useful for generating antibodies against N-glycolylneuraminic acid-containing glycolipids. However, none of the cited references teach or suggest that a mouse with Fas function defects, such as an MRL/lpr mouse, is more useful than a Balb/c mouse administered with LPS, or the like, to generate an antibody against a human protein, which has a high percentage of sequence identity with an endogenous protein of an immunized animal. Applicants submit that an ordinary artisan would have been reluctant to use a mouse having an autoimmune disease to produce the described antibodies if the ordinary artisan was aware at the time of the invention that there is no advantage to using such a mouse when a Balb/c mouse, administered with LPS or the like, is equivalent to a mouse having an autoimmune disease for the generation of antibodies against N-glycolylneuraminic acid-containing glycolipids. Accordingly, the cited references teach away from using mice having an autoimmune disease to generate the described antibodies. Based upon the foregoing, the claims are not obvious in view of the cited references. Accordingly, Applicants respectfully request withdrawal of the rejection.

*Claims 3-9, 14, 16, 18-19, 21, 23 and 25*

Claims 3-9, 14, 16, 18-19, 21, 23 and 25 are rejected under 35 U.S.C. § 103(a) as allegedly being obvious over the '390 publication or the '198 patent, each in view of Lage and further in view of U.S. Patent No. 5,641,488 to Wysocki ("'488"), *see* Office Action, pages 10-11. Applicants respectfully traverse.

Claims 3, 4, 19, 23 and 25 are canceled. Accordingly, the rejection is moot in regard to these claims.

As noted above, the '390 publication and the '198 patent teach away from the instant invention. That is, the cited references do not teach or suggest that a mouse with Fas function defects, such as an MRL/lpr mouse, is more useful than a Balb/c mouse, administered with LPS or the like, for the generation of an antibody against a human protein that has a high percentage of sequence identity with the endogenous protein of an immunized animal. The '488 patent is merely cited for teaching MRL/lpr animals. Specifically, the '488 patent teaches that "[i]n one embodiment of the invention, so-called autoreactive animals, such as mouse strains NZB×SWR(F1) and MRL lpr/lpr animals may be used. "Autoreactive" animals do not require treatment to undergo B cell hypermutation. Such animals need only be immunized with the immunogen of choice when they are in an autoreactive state. Determination of when the animal is in such a state is easily determined by one skilled in the art," *see* column 17, lines 23-30. Applicants submit that this description does not remedy the deficiencies of the '390 publication, the '198 patent and Lage. Based upon the foregoing, the claims are not obvious over the cited references. Accordingly, Applicants respectfully request withdrawal of the rejection.

## CONCLUSION

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Linda T. Parker., Reg. No. 46,046 at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

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Respectfully submitted,

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Attachment: Exhibit A

EXHIBIT A

Annu. Rev. Immunol. 1991. 9:243–69


# *Lpr* and *gld*: Single Gene Models of Systemic Autoimmunity and Lymphoproliferative Disease

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KEY WORDS: *lpr*, *gld*, autoimmunity, MRL, SLE



## *Abstract*

The autosomal recessive *lpr* and *gld* genes induce in mice multiple autoantibodies and the progressive accumulation of large numbers of nonmalignant $CD4^-$ $CD8^-$ T lymphocytes. The clinical syndromes and immune abnormalities associated with these two nonallelic genes are nearly identical and are also highly dependent on background genes. MRL/*lpr* mice are particularly severely affected, and they develop a syndrome that is serologically and pathologically similar to human systemic lupus erythematosus (SLE). Abnormal cell marker expression in the aberrant *lpr* T lymphocytes includes surface antigens normally associated with activated T cells or even with B cells, and it occurs along with enhanced expression of certain oncogenes. The *lpr* gene results in intrinsic abnormalities of both T and B lymphocytes, yet its location and product are unknown. The *gld* gene is located on chromosome 1; its product is also unknown. Although many immunological abnormalities are known, the mechanism whereby these two genes induce autoimmunity and lymphoproliferation remains obscure. Further studies of mice bearing these mutant genes are certain to yield insights into systemic autoimmunity and the control of lymphocyte proliferation.

## INTRODUCTION

*Lpr* and *gld* are presumed single mutations in separate murine autosomal loci (1). Both were originally identified at the Jackson Laboratories and



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have been bred onto several background strains: AKR/J, C57BL/6, C3H/HeJ, MRL/Mp, SJL/J, and BALB/cBy. Homozygosity at either locus results in massive nonmalignant lymphoproliferation, and in autoantibodies and immunopathology similar to those seen in human systemic lupus erythematosus (SLE) and other rheumatic diseases. The *lpr* phenotype, with its impressive accumulation of abnormal T cells combined with high titers of autoantibodies, does not precisely mirror any human disease category. Yet, perhaps better than any other animal model, it mimics SLE both immunopathologically and serologically (2). Thus, the study of *lpr* and *gld* has held the promise of elucidating essential events in the immunoregulation of spontaneous systemic autoimmunity and in the differentiation of lymphoid cells, particularly T cells.



## CLINICAL FEATURES OF *lpr* MICE

Of the strains homozygous for *lpr*, MRL/Mp-lpr/lpr (MRL/*lpr*) has been the best studied. There is 50% mortality by five months in most colonies (2), probably from renal failure and vasculitis. MRL/*lpr* mice develop variable, progressive enlargement of all lymph node groups. A 200-fold increase in cell number compared to control MRL/Mp-+/+ (MRL/+) mice is common. Splenic enlargement also occurs, but to a much less marked degree. Lymphoid infiltrates are seen in many organs, including the salivary and lacrimal glands (3, 4).

In MRL/*lpr* mice, a proliferative glomerulonephritis is seen, with mononuclear cell infiltration, endothelial and mesangial cell proliferation, and crescent formation (5). Immunoglobulin and C3 appear, and anti-DNA is enriched in glomerular eluates. Anti-DNA antibodies with alkaline isoelectric points may be especially injurious due to their charge (6).

MRL/*lpr* mice develop a necrotizing medium-sized arteritis, with involvement of the kidneys, mesentery, and occasionally the coronary circulation (7). Purpuric lesions are frequently visible on the ears and tail, probably reflecting cryoglobulin deposition (8). However, recent investigations have emphasized a granulomatous vasculitis with relatively little immunoglobulin deposition (9, 10).

MRL/*lpr* mice also develop an erosive synovitis (11), although the incidence and extent vary from colony to colony. The arthritis is generally only detectable by a characteristic histopathology, which consists of early proliferation of synovial cells followed by lymphoid pannus formation (12). Its relation to rheumatoid factor levels in individual mice is unclear.

Autoimmune disease in other organs has been reported. MRL/*lpr* mice develop interstitial pneumonitis as well as a pulmonary vasculitis and phlebitis (13). Infiltrative skin lesions together with dermal-epidermal

immunoglobulin deposition and an enhanced sensitivity to UV irradiation (14) are present. There is evidence of muscle inflammation in MRL/*lpr* mice (15), and of blood coagulation abnormalities. Deafness due to cochlear damage has also been reported (16). Elevated amounts of serum amyloid P protein circulate in mice with advanced disease (17).

Manifestations of *lpr*-associated disease on other genetic backgrounds vary widely (18). MRL/*lpr* develops the most severe lymphoproliferation and renal disease and has the shortest life expectancy. C57BL/6-*lpr* (B6/*lpr*), in contrast, develops an indolent autoimmune disease without arthritis, yet has high levels of IgM rheumatoid factor.



## GENETICS OF THE *lpr* DEFECT

*Lpr* appears to be a single autosomal gene which is mainly recessive, although heterozygotes (*lpr*/+) reportedly have some degree of autoantibody production (19, 20) and increased mortality from lymphoid neoplasia (21). The newly described $lpr^{cg}$ gene is allelic to *lpr*; yet unlike *lpr*, it complements the *gld* gene so that $lpr^{cg}/+$, *gld*/+ mice have a lymphoproliferative phenotype (22, 23). The *lpr* gene remains unmapped (24).

The phenotypic expression of *lpr* is strongly influenced by background genes. This is exemplified by variation in degrees of lymphadenopathy, and in the amounts and spectra of autoantibodies seen in different inbred strains homozygous for *lpr* (25). Both lymphadenopathy and antichromatic autoantibodies in the F1 hybrid offspring of MRL/*lpr* and B6/*lpr* mice are pronounced, indicating that the MRL background genes that influence these traits are dominant over the corresponding alleles inherited from C57BL/6 (26). Arteritis and glomulcronephritis appear to be influenced by independently segregating genes (27). The inflammatory arthritis (28) and renal disease (18) of *lpr* mice are also strongly affected by background genes.

The effects of *lpr* are potentiated in the presence of *yaa*, the Y-chromosome gene responsible for BXSB autoimmunity (29), and are greatly reduced in mice also expressing *xid*, a gene related to B-cell maturation (30). B6/*lpr* mice homozygous for the nu/nu mutation do not develop adenopathy, and they acquire only very low levels of autoantibodies (31).

## CELLULAR ABNORMALITIES IN *lpr* MICE

### *General Immune Responsiveness*

The capacity of *lpr* mice to respond to exogenous immune stimulation is impaired. In vitro responsiveness to T-cell antigenic and mitogenic stimuli

is poor (32–36). Despite ongoing polyclonal B-cell activation (37), the antibody response to foreign antigens and polyclonal B-cell activators appears markedly defective (38, 39). Tolerance to protein antigens is harder to establish (40, 41), yet tolerance resistance may not be essential for development of autoimmune disease (42). Delayed hypersensitivity reactions are reduced (43, 44). All of these manifestations of global immunodeficiency in *lpr* mice progress with age.

## *Abnormal Composition of* lpr *Lymphoid Tissue*

The lymph nodes and, to a lesser extent, the spleens of *lpr* mice are subject to progressive infiltration with $CD4^-CD8^-Thy\ 1^+$ cells (double negatives, DN), described in more detail below. Phenotypically normal T cells bearing CD4 or CD8 (single positives, SP) become a progressively smaller fraction of the total cell population so that, in mice with fully developed autoimmune disease, these may constitute 10% or fewer of the cells in the lymph nodes (45). Nonetheless, the remarkable increase in total numbers of lymphoid cells results in a large absolute increase in SP T cells. In addition to the DNs and SPs, the hypertrophied lymphoid tissue of *lpr* mice also contains substantial numbers of $CD3^+$ cells lacking Thy 1 (46).

DOUBLE NEGATIVE T CELLS Some of the most interesting features of the *lpr* disease concern the unusual phenotype and disordered function of the cells infiltrating the lymphoid tissue. The predominant population bears Thy 1, CD3, and low levels of Ly 1 (CD5), but also expresses B220 and certain other B-cell surface antigens (47). Many markers not characteristic of most normal resting T cells are also known to be present on the *lpr* cells, including the J11d heat-stable antigen (48), Ly6C (49), Ly24 (pgp 1) (50), 9F3 (51), Lp-1 (52), 2F3 (53) and PC.1 (54). The *lpr* cells express certain additional markers defined by polyclonal (55) and monoclonal antibodies (56). Another antigen, recognized by monoclonal antibody 100C5, is otherwise seen only on $CD8^+$ T cells and on B cells (57). It has been suggested that the *lpr* DNs can be divided into functional subsets based on the presence or absence of J11d (58). A unique form of $K^+$ channel is found on the membranes of the abnormal *lpr* cells (59).

The DN peripheral cells in *lpr* are apparently phenotypically similar to the $CD4^-CD8^-$ population which predominates in the developing thymus and is also present in smaller numbers even in adult thymus. However, the array of surface markers expressed on *lpr* is clearly different from that of thymic DNs, and distinct differences in functional behavior are observed in vitro (60). In fact, examination of the thymus of *lpr* mice suggests two independent pathways of differentiation, one representing the abnormal development of the *lpr* DNs and the other characteristic of normal thymic

$CD4^-CD8^-$ cells (50). It thus seems likely that the resemblance of *lpr* DN cells to those in the normal thymus is merely superficial.

Although the DN T cells of *lpr* clearly represent an important problem in cell biology, an ongoing conundrum is their significance for autoimmune disease. Are these cells, present in such vast numbers, merely an epiphenomenon without a role in the SLE-like disease? The total number of DN cells in an individual mouse, as measured by combined lymph node weights, correlates poorly with levels of serum immunoglobulin or anti-chromatin antibody (61). Other evidence for dissociation between lymphoproliferation and autoantibody production comes (*a*) from studies of cyclosporin A treatment, which, while drastically reducing the numbers of DN T cells, has surprisingly little effect on autoantibody levels (62), and (*b*) from studies of the effect of MEL-14 antibody (63).

It has repeatedly been observed that isolated DN T cells from *lpr* mice perform poorly, if at all, when their function is tested in vitro (34, 64). Proliferation, interleukin 2 production, and the acquisition of interleukin 2 receptors are markedly deficient after stimulation with mitogens, antigens, and antibody against the T-cell receptor (65). These profound defects can be partly, yet not completely, restored upon incubation of the cells with phorbol ester and calcium ionophore (60). The poor function of the DN T cells may also be related to their lack of CD2 expression (66). This surface molecule is important in the adhesion of T cells to other cells via the LFA-3 ligand, and its absence on *lpr* DN cells may account for a failure to engage in effective cognate cell-cell interactions.

It is not known how *lpr* DN T cells accumulate in vivo; indeed, the fraction of DN T cells undergoing active cell division in peripheral lymph nodes is paradoxically quite low (67). This may be because the liver is the actual site of DN T-cell proliferation (68). Alternatively, the DN T-cell population may undergo expansion at an earlier stage of its development, perhaps as $CD4^+$ or $CD8^+$ T cells.

Whether the *lpr* DN T cells are capable of transition into other cell phenotypes is unknown. Their reported acquisition in culture of broad cytotoxic activity (69) might be due to in vitro differentiation or, alternatively, to the expansion of small numbers of cytotoxic precursor cells. Under short-term culture conditions, stimulation of *lpr* DN T cells with PMA and ionophore does not result in the appearance of CD4 or CD8 (58).

The DN T-cell population shows polyclonal rearrangement of T cell receptor DNA. The overwhelming majority of cells express diminished levels of the $\alpha\beta$ form of the T-cell receptor (66, 70–72), despite high levels of specific mRNA. Cell surface receptor cross-linking results in abnormally rapid modulation (65). Constitutive phosphorylation of the $\eta$21 chain of

CD3, together with a failure to phosphorylate CD3γ, may reflect ongoing activation (73). The poor responsiveness of both *lpr* and normal thymic DN T cells to stimulation with mitogen or antibody to T-cell receptor may be due to inefficient coupling of the T-cell receptor/CD3 complex to functionally normal G proteins (74).

The $V_\beta$ TCR gene family repertoire expressed in *lpr* DN T cells is diverse and reflects appropriate intrathymic negative selection by Mls and I-E (75). $V_\beta 8$ genes are preferentially used, however, particularly $V_\beta 8.3$, which may indicate abnormal positive selection (76, 77; and B. Kotzin, P. L. Cohen, and R. A. Eisenberg, unpublished data). In male MRL/*lpr* mice expressing a transgenic T-cell receptor specific for H-Y plus self, there is decreased intrathymic negative selection of autoreactive T cells (78), as well as abnormal positive selection.

Certain biochemical abnormalities of the DN T cells may be related to their impaired function. For example, turnover of arachidonoyl-PI is markedly increased. It has been proposed that the *lpr* cells have an incomplete form of the phosphoinositol cycle, in which the generation of inositol-containing phospholipid is not linked to the activation of protein kinase C (79). Increased 12-lipoxygenase activity is also observed in *lpr* lymphoid tissue (80).

The disordered cell growth in *lpr* mice may also be related to oncogene abnormalities. Cells from several strains expressing *lpr* have strikingly increased expression of *c-myb*, both at the mRNA and protein levels (81, 82). This abnormality accompanies lymphoproliferation and is associated with increases in *c-myc*, *c-raf*, *c-abl*, and *c-bas* oncogene expression (83). *Lpr* cells have increased phosphorylation of the *fyn* oncogene product, which may be related to activation of the T-cell receptor (84, 85).

A number of cell surface carbohydrate abnormalities exist on the DN T cells. There is aberrant expression of Forssman and Paul-Bunnell antigens (86), and CD45 glycosylation is aberrant (87), with enhanced expression of polylactosaminoglycans. The molecular basis of the latter defect appears to be increased levels in *lpr* $CD4^-CD8^-$ T cells of N-acetylglycosaminyl transferase, which extends polylactosaminoglycans (88, 89). Such surface carbohydrates are normally involved in hematopoietic cell development and activation, and thus may be of considerable importance in the functional abnormalities of the DN T cells.

CD4+ T CELLS Although not as dysfunctional as the DN T cells, *lpr* $CD4^+$ cells are nevertheless deficient in their ability to proliferate and to generate IL-2 and IL-3 in response to mitogenic stimuli (90). A subset of the *lpr* $CD4^+$ cells may act to suppress the helper function of other $CD4^+$ cells in autoimmune *lpr* mice (91). In other work, two $CD4^+$ populations are

described, one of which bears the B220 antigen (92, 93). Although the $B220^-CD4^+$ cells are more responsive to anti-CD3 than to $B220^+CD4^+$ cells, they still are markedly deficient when compared to normal $B220^-CD4^+$ cells. On the other hand, the failure of either CD4-bearing population to show the preferential $V_\beta 8$ usage characteristic of the DN T cells argues against their in vivo differentiation into DNs (94).

Several laboratories have shown that $CD4^+$ autoreactive T cells proliferate when *lpr* lymph node cells are cultured in vitro in the presence of IL-2 (95–97). Long-term $CD4^+$ lines have been established. They are reactive to class-II MHC and are capable of secreting lymphokines which provoke B-cell proliferation and immunoglobulin secretion. It is possible that such $CD4^+$ cells are of importance in the in vivo autoreactive state of *lpr*.

B-CELL ABNORMALITIES IN *lpr* MICE B cells in the *lpr* syndrome have received less attention than T cells. Polyclonal B-cell activation occurs early in disease, as judged by increased numbers of antihapten antibody-forming cells (98). B-cell tolerance to haptenated foreign immunoglobulin is deficient (41, 99), and B-cell surface immunoglobulin capping is abnormally rapid (100). B cells from *lpr* hyperexpress class-II MHC molecules (101). On the other hand, the ability of *lpr* B cells to form colonies in vitro is normal (102), and LPS responses are normal (103).

Much interest has recently arisen concerning the participation of Ly 1-bearing ($CD5^+$) B cells in autoimmunity. The cellular origin of the pathological autoantibodies in mice expressing *lpr* has not been rigorously explored. MRL/*lpr* mice do not, however, have large numbers of cells producing IgM antibody to autologous erythrocytes, suggesting that the $CD5^+$ B cells are not abnormally activated (104). However, IgG antibody forming cells against autologous erythrocytes are described (105).

An intriguing observation not yet understood is the effect of treatment of *lpr* mice from birth with antibodies to IgM. Such manipulation not only averts serological autoimmunity and vasculitis, but also diminishes the degree of lymphoproliferation, suggesting a link between antibody production and the T-cell abnormalities associated with *lpr* (106).

DEFECTS IN OTHER CELL LINEAGES *Lpr* peritoneal macrophages appear to be in an enhanced state of activation (107, 108). Like B cells, they express excessive amounts of Ia (109), possibly due to factors derived from T cells (110, 111). Ia expression on renal tubular epithelial cells is heightened (112). Yet despite enhanced Ia expression, antigen presentation in MRL/*lpr* mice is impaired (113), possibly related to the suppressive effects of $CD8^+$, radioresistant cells (114). In addition, clearance of immune complexes in MRL/*lpr* mice is abnormally prolonged (115, 116). Hepatic non-

parenchymal cell binding of complexes is impaired, even in young mice, suggesting a primary abnormality (104). Mononuclear cells isolated from *lpr* livers are enriched for NK activity (68, 117).

## ABNORMALITIES OF CYTOKINES

Studies on the role of cytokines in the *lpr* autoimmune model have focused on the responsiveness of the DN T-cell population to stimulation by cytokines, on the production of mediators causing B-cell and macrophage activation, and on the potential elaboration of substances that might explain the expansion of the abnormal T-cell population.

For the most part, the DN T cells have proved refractory to stimulation with cytokines. For interleukin 2, this appears to be due to deficient expression of the p75 chain of the interleukin 2 receptor, resulting in a low-affinity receptor (118), although p75 is found by others (119). *Lpr* cells are also defective in their capacity to generate IL-2, but this abnormality is difficult to separate from their global unresponsiveness (120, 121). IL-2 is nevertheless capable of partially replacing T cells in the in vitro production of anti-Sm by MRL/*lpr* T-depleted spleen cells (122); of exerting a stimulatory effect on in vitro generation of antichromatin (123); and of directly stimulating *lpr* B-cell proliferation (124).

IL-3 may be of importance in driving the expansion of the DN population (125), but this is not confirmed (126). The recent observation that an autoantibody to the IL-3 receptor is present in *lpr* sera may be of significance in this regard (127).

Interferon-γ and IL-4 transcripts are elevated in the *lpr* DN T cells (128, 129), and the former has been hypothesized to play a role in the hyperexpression of Ia and consequent autoreactivity of these mice (130). However, administration from birth of a monoclonal anti-interferon-γ antibody has no effect on autoantibody levels, yet it markedly diminishes lymphadenopathy (61). These findings suggest that interferon-γ may be involved in the expansion of the DN population but that it is not critical to autoantibody production.

The T cells of *lpr* are reported to produce a B-cell stimulatory factor, termed L-BCDF (131, 132). Its properties resemble those of IL-4, and its cellular source has not been defined but seems likely to be the $CD4^-$ cells rather than the DNs.

Increased tumor necrosis factor α expression has been found in MRL/*lpr* Kupffer cells (133). Tumor necrosis factor β mRNA is present in increased quantities in MRL/*lpr* kidneys, but whether this is primary or secondary to renal injury is unclear (134).

Interleukin 1 may be of importance in the pathogenesis of some features

of MRL/*lpr* disease. It has been reported that IL-1 expression in renal tissue is increased in this strain, and that a novel mRNA transcript can be detected (135). IL-1 administration enhances the inflammatory arthritis described above (136).

## AUTOANTIBODIES IN *lpr* MICE

The *lpr* gene induces abnormal B-cell activation, which results in striking increases in serum immunoglobulin, particularly IgG, and in the appearance of high titers of a variety of autoantibodies (2). Autoantibody production is most extensive in the MRL/*lpr*, and the SLE-specific antibodies, anti-Sm, anti-ribosomal-P, and anti-Su, are confined to this strain (137–139; and E. Treadwell, P. L. Cohen and R. A. Eisenberg, unpublished). However, fluorescent antinuclear antibody (FANA), antidouble-stranded DNA, and antichromatin antibodies are found in *lpr* homozygous mice with other background genotypes; one specificity, IgM rheumatoid factor, is most prominent in B6/*lpr* (25, 140). In MRL/*lpr*, serum IgG levels reach a mean of 25 mg $ml^{-1}$ at five months of age. This massive hypergammaglobulinemia is accompanied by substantial cryoglobulinemia which appears to involve the IgG3 subclass in particular and may be related to the prominent skin lesions seen in this strain (2, 141, 142). Overall, the picture is indicative of strong polyclonal activation of B cells (98). However, the degree of elevation of titers of certain autoantibody specificities, such as anti-Sm, clearly points to additional specific, probably antigen-driven mechanisms (143).

The spectrum of specificities seen in *lpr* mice is presented in Table 1. Certain of the SLE-specific autoantibodies (anti-Sm, P, and Su) are found

TABLE 1 Major autoantibody specificities in *lpr* mice

| Antigen Recognized | Reference |
|---|---|
| Chromatin (histones) | (200, 201) |
| DNA | (2) |
| Sm | (137) |
| Ribosomal P | (138) |
| Ribosomal S10 | (202) |
| Su | (unpublished) |
| Poly (ADP-ribose) | (203) |
| Mouse IgG | (144, 204) |
| RNA polymerase I | (205) |
| gp70 | (206) |
| Collagen | (207) |

in frequencies in MRL/*lpr* mice similar to those seen in human SLE. In addition, anti-Sm and anti-P are correlated in individual animals (139), as they are in human disease. These findings strongly suggest that the abnormal immunoregulation that leads to autoantibody production in MRL/*lpr* mice must parallel in important ways similar immunoregulatory abnormalities in human SLE. An exception to this parallelism is the presence of IgG rheumatoid factors in MRL/*lpr* mice (144). Although these antibodies are difficult to detect, they can be present in enormous quantities and may be related more to human rheumatoid vasculitis than to human SLE.

The molecular genetic analysis of autoantibodies in *lpr* mice (chiefly MRL/*lpr*) provides important insights (145). Initial studies indicated only that the immunoglobulin gene loci are not abnormal in MRL/*lpr* mice (146), and that the genes found in autoantibody-producing hybridomas derived from this strain are apparently selected from the same pool of genes utilized in normal responses (147). More detailed analysis of particular autoantibody responses in individual mice suggests basic mechanisms of antibody production. For IgG rheumatoid factor, anti-double-stranded-DNA, and anti-Sm, sets of hybridomas derived from individual mice show common clonal origins and apparent somatic mutations in variable region nucleotide sequences (148, 149; and J.-L. Davignon, M. Retter, D. Bloom, D. Pisetsky, P. L. Cohen, R. A. Eisenberg, and S. Clarke, unpublished data). These mutations are relatively clustered in the complementarity determining regions (CDRs). They are usually replacement mutations in the CDRs, while they are disproportionately silent mutations in the framework regions. In some cases, a branched tree showing the deduced in vivo development of an individual clone can be reconstructed. For the anti-DNA response, both somatic mutations and the use of D regions in tandem or in unusual reading frames tend to produce a large number of arginine residues in the CDR3. These positively charged amino acids are presumably important in binding to the negatively charged DNA molecule. In one case, an arginine residue added by somatic mutation changes an antibody's binding from anti-single-stranded-DNA to anti-double-stranded-DNA (150). In all, these findings are reminiscent of secondary responses to exogenous antigens in normal mice and are most compatible with an antigen-driven mechanism for production of these autoantibodies (151).

The overall analysis of gene usage by these autoantibodies from hybridomas derived from *lpr* mice shows that both the light- and heavy-chain genes are drawn from the different gene families in rough proportion to the size of these families in the germ-line repertoire (152). However, several independently derived anti-DNA hybridomas appear to use highly homologous genes, probably representing the same germline gene, as determined

by Southern blots (149). Similarly, in the anti-Sm response, which is overall highly diversified, certain homologous sequences recur in independent clones, suggesting repeated use of the same germ-line genes (J.-L. Davignon, M. Retter, D. Bloom, D. Pisetsky, P. L. Cohen, R. A. Eisenberg, and S. Clarke, unpublished). In fact, in many cases the genes used in the anti-Sm response are highly homologous to genes utilized in the anti-DNA response, again suggesting a nonrandom, recurrent use of certain germ-line genes in an otherwise diversified repertoire. These findings may be a result of the reported cross-reactivity of some anti-Sm antibodies with anti-DNA (153); they may indicate that the anti-DNA and anti-Sm responses are derived from each other in vivo; or they may indicate that certain immunoglobulin variable region genes are more amenable to the production of autoantibodies for reasons other than their antigen specificity, perhaps related to genetic regulatory elements or to idiotype (154).

Crosses between *lpr* strains have shown that the immunoglobulin heavy-chain b allotype locus is used preferentially over the j allotype in $Igh^{j/b}$ heterozygotes. In backcross analysis, anti-Sm, antichromatin, and IgM rheumatoid factor segregate with the b allotype (26). It is not known if this effect is due to $V_H$ or $C_H$ influences.

Nonrandom use of variable region genes is also found in analyses of MRL/*lpr* plasma cells by in situ hybridization with $V_H$-specific probes (155, 156). Individual older animals tend to show repertoires that are skewed toward the use of single $V_H$ families. Although the particular $V_H$ families that are overused in individual mice vary, many mice have repertoires skewed toward J558 (the largest family). A further striking finding is that the severity of renal disease in individual mice correlates with degree of skewing of the repertoire of spontaneously activated splenic plasma cells towards J558. On the other hand, the overall B-cell repertoire, as determined by $V_H$ family analysis of LPS-activated splenic cells, is essentially randomly distributed. The issue of the genetic diversity of the autoantibody response in *lpr* mice has also been investigated by use of anti-idiotype reagents. Anti-idiotypes raised against monoclonal anti-Sm antibodies show the existence of such idiotypes in both *lpr* and normal mice (157, 158). However, the idiotype levels do not appear to correlate with the anti-Sm response, and the structural correlates of these anti-idiotypes or their functional significance in terms of network immunoregulation remains to be demonstrated (154). Similarly, widely cross-reactive anti-DNA idiotypes have been reported and in some cases may be related to recurrent use of the same or similar germline $V_H$ genes (159–161).

The immunoregulation of certain autoantibody responses in MRL/*lpr* mice has been studied in great detail. The anti-Sm and anti-DNA responses

appear to be T-cell mediated, in that in vitro cultures which generate these specificities from spleens from MRL/*lpr* mice require T cells, particularly $CD4^+$ T cells (122, 162, 163). In contrast, the antichromatin response in vitro is not T cell dependent (123). In vivo antichromatin production appears to be antigen-driven, since its major epitopes are located on the outer part of the chromatin particles and are thus easily removed by trypsin digestion (37). Similarly, the anti-Sm response has many characteristics of an antigen-driven response. The addition of Sm antigen to cultures which generate anti-Sm antibodies enhances the in vitro response, whereas anti-Sm antibodies in these same cultures are inhibitory (164). Anti-Sm-positive mice generally recognize at least two of the peptides of the Sm particle (B and D), although in serial bleeds from mice that became anti-Sm positive, antibodies to the D specificity appear first (122). The intensity of the anti-Sm response is several orders of magnitude above background, which implies a specific stimulation, presumably by antigen (165). A curious feature of the anti-Sm response in MRL/*lpr* mice is that only a minority (about 25%) of these genetically inbred animals become anti-Sm positive at five months of age. This distribution is not due to an insensitivity of the assays used to determine anti-Sm positivity, nor is it a result of genetic heterogeneity, maternal effects, or environmental influences. Rather, the distribution appears to reflect stochastic processes in the development of SLE in individual animals (165). Furthermore, when the anti-Sm response appears, the titers increase rapidly and do not show the IgM to IgG progression characteristic of normal responses. Instead, most often the dominant isotype of this response, IgG2a, appears first and soon attains high titers (166). This abrupt time course suggests a positive-feedback mechanism that can accelerate a response once it is initiated. Such a feedback mechanism can be modelled by the administration of passive antibodies to the D Sm peptide, which induces 80% to 100% of MRL/*lpr* mice to become anti-Sm positive. Monoclonal antibodies which recognize both the B and D peptides inhibit rather than enhance the response (167). Overall then, the anti-Sm response in MRL/*lpr* mice resembles an antigen-driven, T-cell response to exogenous antigens.

Anti-Sm antibody responses do not appear spontaneously in other *lpr* strains, such as B6/*lpr* (26, 165). This may be related to a non-MHC-linked, Ir gene effect, which allows MRL/+ but not B6/+ mice to give T cell–proliferative responses after immunization with mouse Sm antigen (168). However, no evidence directly implicates Sm-specific T cells in the spontaneously occurring anti-Sm antibody response. The spontaneous anti-Sm antibody response of MRL/*lpr* mice is also under genetic control. F1 offspring of B6/*lpr* and MRL/*lpr* mice showed a very low incidence of anti-Sm positivity, while MRL/*lpr* × (MRL/*lpr* × B6/*lpr*) backcross mice

are anti-Sm positive at about one half the frequency of MRL/*lpr* mice, suggesting a single recessive gene not linked to MHC (26).

The SLE-specific autoantibodies produced in MRL/*lpr* mice almost certainly reflect the fundamental immunoregulatory abnormalities of this disease. It is not clear what role they play in generating the immunopathological manifestations, such as nephritis and vasculitis. Kidney eluates show some specific concentration of anti-DNA antibodies, and anti-DNA antibodies derived from MRL/*lpr* mice can bind directly to the glomerular basement membrane and can exacerbate ongoing kidney damage (169–171). However, it is unknown how much of the kidney pathology is due to anti-DNA antibodies; other specificities, such as anti-gp70/gp70 immune complexes, may correlate better with severity of disease (172).

## NATURE OF THE *lpr* DEFECT

The biochemical nature of the *lpr* mutation is unknown. It has not been possible to establish any antigenic differences between the *lpr* and +/+. Immunizations between the two strains are unsuccessful or else they produce antibodies that recognize determinants that are not *lpr* specific. There is no evidence for cellular reactivity (mixed lymphocyte reaction) between *lpr* and +/+ mice of the same strain. Given the large number of presumably secondary phenotypic abnormalities that result from this mutation, it is almost certain that only the isolation of the *lpr* gene itself will establish the fundamental nature of the defect. Nevertheless, certain studies involving manipulations of *lpr* mice provide information that permits incisive conclusions regarding the cellular expression of the *lpr* defect, even in the absence of further biochemical or molecular genetic understanding.

The *lpr* gene is expressed in the bone marrow lymphoid stem cells (1, 173, 174). Reconstitution of lethally irradiated *lpr* mice with +/+, T cell–depleted bone marrow abrogates both autoimmunity and lymphoproliferation on the MRL and B6 backgrounds, while reconstitution with *lpr* bone marrow reestablishes the full syndrome. Surprisingly, the converse experiment yields perplexing results. Lethally irradiated +/+ mice reconstituted with *lpr* bone marrow do not show the *lpr* phenotype but, rather, evidence a wasting disease characterized by lymphoid hypoplasia, hepatitis, and pneumonitis with infiltration with large granular lymphocytes, hypogammaglobulinemia, and early mortality (175). Although this syndrome has been compared to the graft-vs-host reaction, its mechanism is probably different since there is no evidence of "histoincompatibility" between *lpr* and +/+; and the wasting syndrome does

not require the transfer of mature T cells, since rigorous T-cell depletion of the transferred bone marrow or the use of fetal liver does not change the overall result (174). Nevertheless, the maturation of donor *lpr* T cells is necessary, since athymic (nu/nu) +/+ recipients of *lpr* marrow remain surprisingly healthy (176). The interpretation of these experiments is even further complicated by the finding that implantation of *lpr* spleen under the kidney capsule of +/+ mice can transfer the *lpr* phenotype without engendering the wasting syndrome (177). Overall, there appears to be an important expression of the *lpr* gene in the nonlymphoid radioresistant cell populations, as well as in the bone marrow precursors. This possibility is supported by recent experiments indicating that *lpr* lymph nodes transplanted into irradiated +/+ recipients that have received *lpr* bone marrow did show local hypertrophy (178).

Additional cell-transfer experiments have further defined the cellular expression of the *lpr* defect. The lymphoid precursors of *lpr* bone marrow, either transferred or remaining after apparent lethal host irradiation, have a preferential ability to proliferate compared to +/+ bone marrow and will replace +/+ marrow in its lymphoid elements, but not erythroid elements, in chimeric mice (179, 180). Such extreme *lpr* bone marrow takeover is not seen in other chimera experiments in C57BL/6 congenic mice (181). In this approach, *lpr* and +/+ T cell–depleted marrows, distinguished by their Ly-1 allotypes, are transferred to irradiated *lpr* recipients. Five months post transfer, the thymic and peripheral blood T-cell populations are relatively well balanced between the *lpr* and +/+ bone marrow progeny, indicating equal chimerism. In contrast, the abnormal phenotype T cells that predominate in the hypertrophied lymph nodes are entirely of *lpr* origin. These experiments prove that the expansion of an abnormal population of DN T cells in *lpr* mice requires the expression of the *lpr* gene in the abnormal T cells themselves. This population cannot result from the effects of other *lpr*-determined external stimuli. Further experiments address the role of the *lpr* gene in the production of autoantibodies (182). *Lpr* and +/+ bone marrows, marked by immunoglobulin heavy chain allotypic differences, are transferred to irradiated *lpr* mice. Dual chimerism is verified by the presence of IgM- and IgD-bearing cells of both allotypes in the peripheral blood and spleen. Nevertheless, IgG2a autoantibodies are entirely of the *lpr* allotype. Therefore, the spontaneous autoantibody production characteristic of SLE in *lpr* mice requires the expression of the *lpr* gene in the autoantibody-producing B cells. It cannot occur merely as the result of abnormal, *lpr*-determined T-cell help being delivered to an essentially normal B-cell population.

The thymus is also essential in the MRL/*lpr* syndrome, as neonatal thymectomy substantially inhibits the development of lymphadenopathy

and autoimmunity (183–185). Thymectomy later in life (three to six weeks) has a decreasing inhibitory effect on disease expression, which suggests that the massive lymphoid accumulation found at ages three to five months occurs mainly through peripheral proliferation. These studies can be extended to show that neonatally thymectomized MRL/*lpr* mice reconstituted with either *lpr* or +/+ thymus develop the full *lpr* phenotype (186). This indicates that the *lpr* defect is not expressed in an essential way in those thymic elements that are responsible for T-cell development and differentiation. An essential role for T cells in the *lpr* syndrome is further demonstrated by in vivo treatment with anti-CD4 or anti-Thy-1 monoclonal antibodies (187, 188). In both cases, the lymphoproliferation is substantially inhibited and autoantibody production is decreased. The anti-CD4 results are perhaps somewhat surprising, since the T cells that accumulate in the largest numbers in *lpr* mice do not bear this marker. Either the DN T cells pass through a $CD4^+$ stage (a conclusion further enforced by the appropriate negative selection of T cells bearing certain autoreactive receptors, see above); or $CD4^+$ cells influence the proliferation of *lpr* DN T cells. In either case the *lpr*-driven B-cell autoantibody production is T-cell dependent. However, none of the information currently available demonstrates whether T cells unique to *lpr* are necessary.

## *Gld* MICE

The *gld* (generalized lymphoproliferative disease) mutation arose in the inbred C3H/HeJ strain of mice at the Jackson Laboratories (1, 189). Like *lpr*, *gld* is autosomal and recessive, and leads to lymphoproliferation and autoimmunity. The phenotype of C3H/*gld* mice has so far been nearly indistinguishable from that of the C3H/*lpr*, which suggests that the two mutations affect the same pathways of lymphocyte differentiation. However, *lpr* and *gld* are clearly not allelic, and they do not complement each other, in that double heterozygotes are essentially normal. The *gld* mutation maps to the long arm of chromosome 1, close to the At-3 RFLP (190).

Clinical aspects of the *gld* phenotype have been found to reproduce that of the *lpr* gene on the same C3H background (189). C3H/*gld* mice develop massive lymphadenopathy and a lesser degree of splenomegaly over the first three to five months of life. Concomitantly, they show hypergammaglobulinemia, with particularly high expression of the IgG2a isotype; and high titers of antinuclear antibodies, including antibodies to double-stranded DNA, as demonstrated by the Crithidia luciliae assay. They have mesangial deposition of immunoglobulin, but little clinical nephritis. As in the *lpr*, C3H/*gld* male mice that express *xid* have diminished

autoantibodies but no change in lymphoproliferation (191). Attempts to transfer the cells from the hypertrophied *gld* lymph nodes show no indication of malignancy.

The phenotype of the *gld* T cells is also strikingly similar to that seen in *lpr*. Most cells are CD4$^-$, CD8$^-$, CD2$^-$, IL-2R$^-$, and sometimes even Thy-1$^-$, while they are B220$^+$, Ly-1$^+$, Ly-6C$^+$, PC-1$^+$, Ly-22$^1$, Ly-24$^+$, and variably positive for J11d, Lp-1, and Lp-2 (46, 49, 66, 192). They express several lectin-binding sites normally associated with B cells. The *gld* T cells bear CD3 and TCR$\alpha\beta$, although with diminished surface concentration (193), combined with enhanced cellular messenger RNA (70, 194). The T-cell repertoire of *gld* shows the expected clonal deletions associated with induction of tolerance to Mls and MHC determinants, as well as overall skewing to the $V_\beta$8 family (46, 195, 196). The CD3 complex has abnormal constitutive tyrosine phosphorylation of the $\eta$21 chain, combined with a failure of serine phosphorylation of the $\gamma$ chain in response to exogenous stimuli (73). The T cells hyperexpress p59$^{fyn}$ (84), as well as other oncogenes, including *myb*, *raf*, and *abl* (83). Finally, *gld* DN T cells also show the functional hyporesponsiveness of *lpr* DN cells, as they do not respond in mixed lymphocyte culture, or to Con A or to Ly-6C, nor do they produce IL-2 or MHC-directed CTL (193, 197). They also share the unusual potassium channels characteristic of the *lpr* DN T cells (198).

The abnormal T cells that characterize the *gld* mouse thus probably arise in the same way as do the *lpr* DN T cells. Their differentiation apparently involves a CD4$^+$ stage, since the specific $V_\beta$ deletions they undergo normally require expression of this marker (199). Recent work (196) has also suggested that they pass through a CD8$^+$ stage, since their CD8 gene is hypomethylated (a result not yet duplicated for the *lpr*). A unique, but striking, difference between *lpr* and *gld* arises in a stem cell transfer experiment. Reconstitution of irradiated C3H/+ mice with C3H/*gld* bone marrow results in a *gld* phenotype (196), while *lpr* transferred to +/+ produces the unusual wasting syndrome described above, at least on the B6 and MRL backgrounds.

## CONCLUSIONS

The *lpr* and *gld* mouse strains are important models for human autoimmunity, particularly SLE. They provide insights into mechanisms of immunopathology, involving the skin, arteries, and kidneys. They are highly useful models of spontaneous, generalized autoantibody production, and they have led to increased understanding of cellular and molecular genetic control of these abnormal responses. At the same time, the study of the extraordinary abnormal lymphoid hyperproliferation is an im-

portant impetus for understanding thymic and extra-thymic T-cell differentiation and the potential roles of cytokines in autoimmune disease.

Despite the extensive work on these strains over the last 15 years, essential major questions remain unanswered. The exploration of these issues in our opinion will constitute the most important directions in this area in the near future:

1. What are the *lpr* and *gld* genes? Only the isolation and characterization of the mutant genes themselves will fully elucidate how these single mutations can have such profound effects on humoral and cellular immunity.

2. What is the nature of T-B collaboration for autoantibody responses? Except for the fact that T cells are required, surprisingly little is known in this area. Are *lpr* T cells required? Is T-B collaboration cognate (requiring direct recognition of B cells by T cells)? Is T-B collaboration MHC restricted? What is the specificity of the T cells that help B cells for autoantibody production? Are they directed against the same autoantigens that the B cells see; against other autoantigens or foreign antigens that are complexed with the B-cell antigen; or against structures on the B cell itself, e.g. Ia or immunoglobulin?

3. What is the role of autoantigen? Why does the spectrum of SLE autoantibodies include particular specificities and not others? How are these antigens seen? Do they come from dying cells? Are they presented "inside out" by live cells?

4. What is the role of background genes? Why is the MRL/*lpr* strain so much more autoimmune than strains with *lpr* on other backgrounds? What genes are contributing to this background effect?

5. How do the *lpr* and *gld* DN T cells arise? Are they an expanded normal population? Where do they proliferate? Why do they accumulate in such abnormal numbers? What role do they play in the clinical disease or in autoantibody production?

6. How are the *lpr* B cells abnormal? Why are they particularly susceptible to autoantibody production?

7. How are the clinical manifestations of the disease produced? What roles do autoantibodies play in the generation of tissue destruction? Are there other important effector mechanisms, such as T cells, NK cells, etc?

8. Why do certain immunoglobulin genes preferentially contribute to autoantibody production? What is the role of individual variable regions or an entire Igh locus (b allotype)?



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